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Effects of parathyroid hormone dosage and schedule on bone regeneration

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Abstract: This study investigated the effects of administration of parathyroid hormone (PTH) at different dosages and schedules on bone regeneration in critical-size bone defects in rat calvariae. After calvarial defects had been prepared in 50 rats, they were divided into five treatment groups: 15 µg/kg PTH daily (PTH-15), 35 µg/kg PTH three times per week (PTH-35), 105 µg/kg once per week (PTH-105-1), 105 µg/kg three times per week (PTH-105-3), and controls given vehicle alone. Bone regeneration was evaluated radiographically using micro-computed tomography (micro-CT) or histologically. The amount of newly generated bone in the calvarial defects was found to be significantly greater in the PTH groups than in the control group, and did not differ significantly among the PTH-15, PTH-35, and PTH-105-1 groups, whereas the PTH-105-3 group showed a significantly greater degree of new bone formation than the other PTH groups. It appeared that a higher dose of PTH stimulated a greater degree of bone regeneration in this experimental setting. The present results also suggest that the total dose of PTH administered is significantly related to the amount of bone regenerated within a defined period, indicating that less frequent administration of PTH might be a feasible protocol for bone regeneration therapy.

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Keywords: parathyroid hormone; administration protocol; bone regeneration therapy; rat calvaria; micro-CT.

Introduction

Various clinical protocols for regeneration of bone tissue have been introduced (1,2). However, few of them have been effective for treatment of severe alveolar bone loss in patients with periodontal disease.

Parathyroid hormone (PTH) is a major regulator of bone remodeling and calcium homeostasis, exerting both anabolic and catabolic effects in bone tissue. Continuous treatment with PTH causes bone resorption, whereas intermittent PTH treatment promotes bone formation (3). Injection of rats with PTH accelerates the differentiation of osteoprogenitor cells into osteoblasts (4). Administration of PTH also inhibits osteoblast apoptosis, causing prolonged, enhanced bone formation (5). The anabolic effects of intermittent PTH administration are beneficial in patients with severe osteoporosis, increasing bone mass and reducing the incidence of fractures (6).

Recently, the possible utilization of PTH for regenerative applications, such as fracture healing and treatment of osseous defects, in the craniofacial region has attracted interest (7). However, little information on the reparative effects of PTH at various dosages for various periods on bone defects has been available. Therefore, the purpose of the present study was to evaluate the effects of various schedules and dosages of PTH on the regeneration of critical-size bone defects in rat calvaria.

Materials and Methods

Animals

Fifty 12-week-old male Fischer rats weighing 250-300 g were used. The animals were housed in metal cages in an

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animal room (temperature 22°C, 55% relative humidity, 12/12-h light/dark cycle) and fed a standard laboratory diet and water. This study was approved by the Animal Experimentation Committee of Nihon University School of Dentistry, Japan (AP13D018).

PTH

Synthetic human PTH (1-34 PTH; Asahi Kasei Pharma, Tokyo, Japan) was dissolved in 0.1 M Tris-HCl, at pH 7.5, containing 2% bovine serum albumin.

Surgical procedure

The animals were premedicated by inhalation of isoflurane and then general anesthesia was induced by intraperitoneal injection of a mixture of 0.15 mg/kg dexmedetomidine hydrochloride, 2.0 mg/kg midazolam, and 2.5 mg/kg butorphanol tartrate. The forehead of each rat was also locally anesthetized with an injection of 0.5 mL of 1:8-diluted lidocaine (Xylocaine; Astra Zeneca, Osaka, Japan). The dorsal cranium was shaved and prepared for surgery aseptically. A horseshoe-shaped skin incision was made over the head, the parietal area was exposed under aseptic conditions, and the periosteum was elevated to expose the bone. Using a trephine burr constantly cooled with sterile saline, critical-size (5.0-mm diameter) bone defects were created on the right side of the parietal bone to avoid the sagittal suture (Fig. 1). The resulting calvarial disk was then removed carefully to avoid tearing the dura. After thoroughly rinsing the area with physiological saline to wash out any bone fragments, the skin was closed using 4-0 silk (Ethicon, Somerville, NJ, USA).

PTH administration

The treated rats were divided into five groups, which received PTH by subcutaneous injection at 15 µg/kg daily (PTH-15), 35 µg/kg PTH three times a week (PTH-35), 105 µg/kg PTH once a week (PTH-105-1) or 105 µg/kg PTH three times a week (PTH-105-3). The control animals were given only vehicle (sterile saline) each day during the experiment (Fig. 2). The first day of PTH administration and surgery was designated as day 0.

Measurement of serum calcium (Ca²⁺) concentration and alkaline phosphatase (ALP) activity

Under ether anesthesia, ~80 µL of blood was obtained from the subclavian vein every day for one week after surgery. The blood was centrifuged and the serum was stored at -80°C. Total serum Ca²⁺ and ALP were determined using a detection kit with automated clinical chemistry analyzers (7180 automated analyzer, Hitachi,

Fig. 1 Critical-size calvarial bone defects (5-mm diameter) were created in the dorsal part of the right parietal bone.

Fig. 2 Schedule of PTH administration.

Ibaraki, Japan).

Micro-computed tomography (micro-CT) analysis

A micro-CT apparatus (R_mCT; Rigaku, Tokyo, Japan) was utilized to take images on days 0, 7, 14, 21, and 28 to examine bone regeneration *in vivo*. The images were reconstructed on a personal computer using the *i-View* software package (Rigaku, *i-View* Image Center, Tokyo, Japan). On day 0, a screen cylinder was drawn to define the initial bone defect. The bone volume (BV) was measured as the bone tissue within the cylinder on voxel images using BV measurement software (Kitasenju Radist Dental Clinic, *i-View* Image Center, Tokyo, Japan), which calculates the gray values and corresponding number of voxels in the regions of interest.

Histological and histometric analyses

The animals were sacrificed at 28 days after surgery. The skin was dissected, and the defect sites were removed,

Fig. 3 Schematic drawing of regenerative bone in calvarial defects and histometric parameters.

Fig. 4 Concentration of serum Ca²⁺.

Fig. 5 Activity of serum ALP.

along with the surrounding bone and soft tissues. The specimens were then fixed in 10% neutral buffered formalin, decalcified in 10% EDTA for one week, and embedded in paraffin. Coronal sections (5 μ m thick) were prepared through the center of each circular defect, stained with hematoxylin and eosin, and examined under a light microscope equipped with a morphometric system

(Model BHF-142; Olympus, Tokyo, Japan).

Closure of each bone defect was determined by measuring the distance between the defect margins, and was expressed as a percentage of the width of the original bone defect. The osteoblast-like cells were counted in four areas at the edge of the original defects and expressed as averages (Fig. 3).

Statistical analysis

Means and standard deviations were calculated for the reossification ratio on days 7, 14, 21, and 28. The mean BV, defect closure, and the numbers of osteoblasts-like cells were compared among the groups using the Kruskal-Wallis rank test. The level of statistical significance was set at 0.05. Statistical analyses were performed using the SPSS software package (Ver. 16.0J for Windows; SPSS, Inc., Chicago, IL, USA).

Results

Concentration of serum Ca²⁺ and activity of serum ALP

There were no significant differences in the serum Ca²⁺ levels among the PTH and control groups. The PTH groups tended to have higher serum ALP levels than the control group, but these differences were not significant (Figs. 4, 5).

Micro-CT analysis

In the micro-CT images, the radiopacity increased gradually in a time-dependent manner in all groups. The outline of the bone defect in the PTH groups was blurred and an irregular high-density region was observed in the defect area. In the PTH groups, there was significantly greater radiopacity at 21 and 28 days, compared with the control group (Fig. 6).

The BV was significantly higher in the PTH groups than in the control group at days 21 and 28. The BV did not differ significantly among the PTH-15, PTH-35, and PTH-105-1 groups, but the PTH-105-3 group had significantly more new bone at day 28 (Fig. 7).

Histological and histometric observations

The PTH groups showed significant formation of new bone throughout the defects, relative to the control group. The PTH-105-3 group showed considerably thick lamellar bone. The control group showed limited bone formation with fibrous connective tissue (Fig. 8).

The closure of bone defects was enhanced significantly in the PTH groups relative to the control group. The PTH-105-3 group exhibited significantly greater defect closure than the other groups (Table 1).

Fig. 6 Micro-CT images of critical-size bone defects at days 7-28: (a) Control, (b) PTH-15, (c) PTH-35, (d) PTH-105-1, and (e) PTH-105-3.

Fig. 7 Regenerative bone volume in critical-size bone defects. The same lower case letters on the same days indicate no statistical difference, Kruskal-Wallis rank test ($P > 0.05$).

Fig. 8 Histological sections of critical-size bone defects at 28 days. The boxed area is the high-magnification histology. (a) Control, (b) PTH-15, (c) PTH-35, (d) PTH-105-1, and (e) PTH-105-3.

More osteoblast-like cells were observed at the edge of the original bone defect in the PTH groups. The greatest number of osteoblast-like cells was present in the PTH-105-3 group (Table 2).

Discussion

Intermittent administration of PTH has an anabolic effect, promoting the healing of bone defects. The results of this study indicated that the amount of newly generated bone in critical-size bone defects was significantly higher, and bone defect healing was faster, in the PTH groups than in the control group.

Various dosages of PTH have been used to promote regeneration of new bone. PTH administered at 60 $\mu\text{g/kg}$ was reported to increase bone deposition in rat parietal critical-size bone defects (8). Moreover, PTH

at 25 $\mu\text{g/kg}$ accelerated new bone formation in rabbit mandible critical-size bone defects (9). Yun et al. (10) and Stancoven et al. (11) showed that PTH administration at 15 $\mu\text{g/kg}$ increased bone formation in rat calvarial bone defects. Accordingly, the lowest reported dose of PTH, 15 $\mu\text{g/kg}$, was used in the present study. However, the optimal dose and schedule for enhancing bone defect healing remain unclear. In the present study, bone regeneration was enhanced significantly in the PTH-105-3 group relative to the other three groups, PTH-15, -35, and PTH-105-1, suggesting that a greater dose of PTH stimulated regeneration of more bone in rat critical-size bone defects. In contrast, there were no significant differences in bone regeneration among the PTH-15, -35, and PTH-105-1 groups. Because the cumulative dosage of PTH administered to these three groups was the same, it

Table 1 Percentage of bone closure rate

Group	Closure of bone defects (%)
Control	10.3 ± 7.3 ^a
PTH-15	40.3 ± 15.2 ^b
PTH-35	48.9 ± 12.4 ^b
PTH-105-1	55.3 ± 4.2 ^b
PTH-105-3	66.2 ± 9.3 ^c

The values with the same superscript letters indicate no statistical difference, group means ± SD, Kruskal-Wallis rank test ($P > 0.05$)

could be considered that the total amount of PTH delivered during the experimental period is a significant factor for regenerative bone healing.

Daily intermittent subcutaneous administration of PTH has been reported to enhance bone regeneration (12,13). However, as daily injection of PTH would be stressful to patients, an effective schedule for optimal PTH administration needs to be considered. The amount of bone regenerated in the PTH-15, -35, and PTH-105-1 groups was approximately the same, but the total frequency of PTH administration differed among the groups. In other words, a lower frequency of PTH administration using a larger dosage, and a higher frequency with a small dosage might be both equally effective for achieving a given degree of bone regeneration, as long as the total cumulative amount of PTH is the same. This concept would be useful when considering a better schedule of PTH administration.

PTH is a peptide hormone that regulates calcium and phosphate metabolism. Continuous infusion of PTH has been reported to induce catabolic effects in bones, resulting in hypercalcemia (14). In the present study, blood Ca^{2+} levels did not increase in the experimental groups relative to the control group, suggesting indicates that the dose and frequency of PTH administration in this study exerted anabolic effects. On the other hand, serum ALP levels showed a tendency to increase, suggesting active bone turnover, as the two are known to be correlated with each other (15).

Our histological observations showed that PTH stimulated new bone formation with an increase in the number of osteoblasts around the existing bone. A previous study had suggested that this increase in the number of osteoblasts was not dependent on the proliferation of osteoprogenitor cells or osteoblastogenesis; rather, PTH affected existing cells (16). These lining cells might undergo transition to an active form and resume matrix synthesis in response to PTH treatment.

In conclusion, the present findings suggest that the greater the dose of PTH, the more bone is regenerated

Table 2 Number of osteoblast-like cells observed around the edge of the existing bone

Group	Number of cells
Control	70 ± 8 ^a
PTH-15	121 ± 30 ^b
PTH-35	146 ± 22 ^b
PTH-105-1	129 ± 29 ^b
PTH-105-3	244 ± 35 ^c

The values with the same superscript letters indicate no statistical difference, group means ± SD, Kruskal-Wallis rank test ($P > 0.05$)

in critical-size rat calvarial bone defects, and that the total dose of PTH administered within a defined period is significant for bone regeneration. Therefore, a lower frequency of PTH administration with a higher dose could be a possible protocol design for bone regeneration therapy.

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